TIPS, TRICKS & TECHNIQUES

STEAM Connections: Painting with Bacteria

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Abstract

Arts-related science activities provide unique opportunities to engage students' strengths and motivate different types of learners (Jolly, 2014). Incorporating arts into the discussion of gene expression and microbiology introduces students to a multidisciplinary approach to STEM and provides an opportunity to explore the use of science in different fields such as design, art, and industry. In this protocol extension students create living works of art on agar plates by "painting" with E. coli that express fluorescent proteins of various colors.

Key Words: *painting; bacteria;* E. coli; *protein; gene expression; DNA.*

○ Introduction

It is all too common for students to think of art and science as being entirely separate and unrelated. Incorporating arts into the discussion of gene expression and microbiology introduces students to a multidisciplinary approach to STEM and provides an opportunity to explore the use of science in different fields such as design, art, and industry. Arts-related science activities also provide unique opportunities to engage students' strengths and motivate different types of learners (Jolly, 2014). In this activity, students create living works of art on agar plates by "painting" with *E. coli* that express fluorescent proteins of various colors.

This activity is designed for grades 8–12 and supports students in making the connection between DNA and gene expression. However, it can also be modified for younger students by emphasizing the role of microorganisms such as bacteria in research and in their lives. Undergraduate audiences can learn about plasmid construction and bioinformatics by focusing on the DNA sequences of the plasmids involved.

Bacterial transformation is a powerful research tool as well as a classic classroom laboratory activity that allows students to experience and reflect on core disciplinary ideas in biology. This technique provides a simple and effective demonstration of how DNA contains information for the production of proteins and how changes in DNA can alter the phenotype of an organism (HS-LS1. A, NGSS, 2013). At the Fred Hutchinson Cancer Research Center's Science Education Partnership (SEP), teachers have been using a set of fluorescent plasmids to practice basic bacterial transformation with their students. However, many teachers have extended their lessons by using the transformed bacteria to paint with, thereby reaching different learners and reinforcing the concept that genetic engineering can create a specific protein product.

In research, plasmid design is an essential tool of molecular biology. Engineered plasmids are used in pharmaceutical development and to study gene expression and disease pathology. The ability to add new genetic instructions into a cell and expect reliable and reproducible protein expression has led scientists to develop new tools like CRISPR Cas-9 for genome editing, and to create cellular human insulin factories using bacteria and yeast. Scientists creating new plasmid constructs often include genes for antibiotic resistance or fluorescence as a visual indication of positive bacterial transformation.

The plasmids used in this activity were designed by the Tsein Lab at the University of California San Diego (Figure 1). Transformed bacteria containing the fluorescent plasmids can be ordered from the nonprofit plasmid repository Addgene (see Table 1) (Shaner, 2004). The sequences for these plasmids are also available on the Addgene website. In the activity described in this article, students take advantage of the introduced fluorescence genes to create living paintings with bacteria, forming a more tangible connection between gene expression and phenotypic changes.

○ Preparation

It is not necessary to do a bacterial transformation in order to do the painting, since the bacteria arrive containing the plasmids already. The Addgene plasmids listed in Table 1 are sent in bacteria stabbed into an agar slant. The bacteria should be streaked for isolation onto an LB (Luria Bertani or Lysogeny Broth) agar plate containing ampicillin as soon as possible. After overnight incubation,

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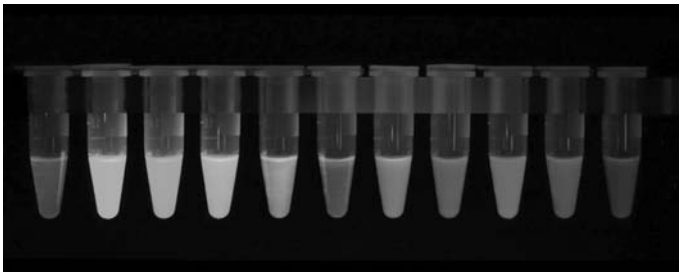

Figure 1. Composite of fluorescent cells from the Tsien Lab at the University of California, San Diego (http://www.tsienlab.ucsd.edu/Images.htm).

Table 1. Plasmids yielding fluorescent proteins, available from Addgene (www.addgene.org).

Plasmid Name	Visible Color	Color Under UV
pNCS mCherry, #91769	Dark Pink	Dark Pink
pNCS mHoneydew, #91760	Yellow/Lime	Lime
pNCS mTangerine, #91763	Pink	Orange/Pink
pNCS BFP, #91757	White	Blue
pNCS Venus (YFP), #91759	Yellow	Yellow/Lime

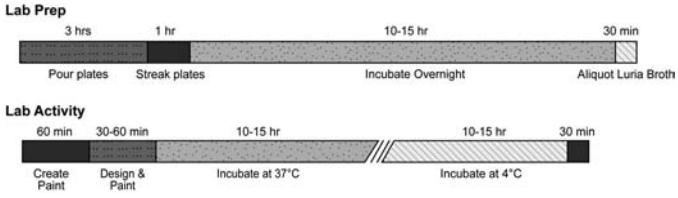

Figure 2. Timeline.

single colonies can be selected and used for creating the bacteria paint or prepped to isolate and purify the plasmid for use in a transformation protocol (Figure 2).

If you are interested in doing transformation as a lab activity, you will first need to do a "miniprep" to isolate the plasmids from the bacteria sent by Addgene. Commercial miniprep kits are available; most require high-speed centrifugation, so be sure to check the specific protocol beforehand. Students can then insert those plasmids into non-transformed bacteria using a transformation protocol (see Extra Resources). Calcium chloride and heat shock are used to introduce the plasmids into competent bacteria. K-12-safe *E. coli* strains JM101 and NEB5-alpha (available to teachers through New England BioLabs) can be used for all the plasmids listed here. Once transformed, the bacteria are plated on ampicillin agar plates and incubated overnight. The colonies of transformed *E. coli* will produce visible color and fluorescent color under UV light (Table 1). Over time, the colonies will become more vibrant as the proteins are continually produced within the cells. Students can then select successfully transformed colonies to use as their paints.

○ Materials

- Stock plates of transformed bacteria
- Sterile toothpicks
- Microcentrifuge tubes
- Micropipet & tips
- Sterile Luria Broth
- Sterile LB/amp plates (for stock)
- Sterile LB plates (for painting)
- Ampicillin
- Disinfectant (10% bleach or 70% alcohol)
- 37°C incubator (egg incubators work well)
- Autoclave — if making your own LB agar & Luria broth

○ Preparing the Paint and Plates

Pour LB/ampicillin stock plates and LB plates for students to use as canvases. Make at least two stock plates for each plasmid of the transformed bacteria by streaking LB/ampicillin plates to create isolated colonies. To create the medium for the bacteria paint, add ampicillin (100 mg/ml) to sterile Luria Broth at 1:1000 (1 µl ampicillin to 1 ml of Luria Broth). Transfer ~500 µl of the solution into microcentrifuge tubes. Store tubes in the refrigerator until ready to use. The ampicillin is light sensitive, so use your solution soon after preparation.

To create their paint, the students can select the best colony with a sterile toothpick and transfer it to a microtube of Luria Broth with ampicillin. They then suspend the colony in the Luria Broth using a pipet or vortexer, and incubate the tubes for 20–30 minutes at 37°C to allow the bacteria to multiply.

○ Painting with Transformed Bacteria

To plan their designs, students first make a template by tracing around the outside of an empty petri dish onto a piece of paper. Simple line drawings and block images work best. In general, images with recognizable shapes (flowers, mountains, neurons) and without backgrounds produce better results. Have students flip over their plates and tape their designs face-up onto the bottom so the image is visible through the agar. Discuss the limitations of bacterial paint, such as the inability to blend colors, the potential for contamination, and different growth rates that can occur on the same plate.

Toothpicks are the easiest way to paint the bacteria onto a plate. Students dip the rounded end of a sterile toothpick into a tube of bacteria-LB/ampicillin paint and apply it to their agar plate. Wooden toothpicks are cheap! If a tip appears jagged, select a new one. Encourage students to use new toothpicks often when painting with a particular color, and to always use a new toothpick when beginning a new color to avoid cross-contamination. The paint does not need to be heavy, and students should try to avoid tearing into or puncturing the agar. Cotton swabs generally take up too much of the paint solution, making precision difficult and greatly increasing the chances of contamination. Brushes can be used, but they should be rinsed in a 10 percent bleach solution and then in sterile water in between colors.

Place the plates agar side up in a darkened 37°C incubator overnight (wrap with aluminum foil if light is an issue). After 24 hours, the plates should have visible bacterial growth as their art comes to life. The bacteria will glow under UV light but should also express some color under normal light. Although colors will be visible after the overnight incubation, for better results place the plates in a refrigerator (4°C) for 24 hours after incubation. This allows the bacteria to express more of the fluorescent proteins, creating more vibrant colors. When the incubation time is completed, students can use cell phones to photograph their artwork. Teachers can provide a cardboard box to create a hood, as well as a digital camera and tripod to aid in photography. Students may need to experiment to find the best way to visualize fluorescence. A UV light under the plate will give different results than one held over the plate.

By using bacteria as paint, students can learn about gene expression and how plasmids are used by molecular biologists as a tool for harnessing a cell's genetic machinery. This extension to the transformation protocol is also an opportunity to further discuss the use of fluorescent proteins as a tag to help identify successful plasmid incorporation, or as an example of employing an organism to produce a desired protein. Incorporating art and science can be a useful mechanism for engaging different learners, introducing cross-disciplinary skill, and fostering creative and critical thinking in students.

○ Sample of Student Response to Activity

"The project created a wonderful example of real world application of genetics. It was fun to work hands-on with the bacteria and as a student who enjoys drawing, it was great to have an excuse to use my artistic skills in science class. It was so rewarding to spend the time working with the bacteria and then have the work produce a final result that was both entertaining, and memorable." (Miranda, Class of 2020)

"I really enjoyed the bacteria art project because not only did it help me understand how bacteria can replicate and pass down the florescent protein DNA sequence, but it also helped wrap my head around the massive quantity of bacteria produced. It was also a very fun activity that allowed me to be creative while learning." (Conor, Class of 2020)

"It was really fun not only drawing with bacteria (which is super cool and not many people can say they have done it) but also an interesting experience making, and growing them. I think that it is a really fun way to teach students a complicated concept with many steps through an interactive and fun activity and really helped me understand it a lot better." (Albert, Class of 2020)

○ Extra Resources

- Addgene Roger Tsien Repository (https://www.addgene.org/Roger_Tsien/)
- SEPGuides pFLO Transformation Protocol (http://www.fredhutch.org/en/education-training/sep/teachers/labs-lessons.html)
- ScienceBridge Transformation Resources (http://sciencebridge.ucsd.edu/programs/labs/content-areas/transformation.html)
- American Society for Microbiology, Agar Art Contest (https://www.asm.org/index.php/public-outreach/agar-art)
- New England BioLabs Inc. (https://www.neb.com)

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